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COMMUNICATION

Use of a hydrogel polymer for reproducible surface enhanced Raman optical activity (SEROA)†‡

Saeideh Ostovar Pour,*[a] Steven E. J. Bell[b] and Ewan W. Blanch[a]

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We present surface enhanced Raman optical activity (SEROA), as well as Raman, SERS and ROA, spectra of D- and L-ribose. By employing a gel forming polyacrylic acid to control colloid aggregation and associated birefringent artefacts we observe the first definitive proof of SEROA through measurement of mirror image bands for the two enantiomers.

As a result of its sensitivity to chirality, Raman optical activity (ROA), which measures a small difference in the intensity of vibrational Raman scattering from chiral molecules in right- and left-circularly polarized light,[1,2] is a powerful probe of the structure and behaviour of biomolecules in aqueous solution.[3–7] However, ROA is a very weak effect being ~3–5 orders of magnitude smaller than the parent Raman scattering. The conditions of high concentration and long data collection time required for ROA currently limit its application for a wide range of biological samples. These limitations could possibly be overcome using the principles of surface enhanced Raman scattering (SERS)[8–10] in which a sample in the presence of surface plasmons localized on a neighbouring nanostructured feature of a metal surface can interact with the incident light leading to large enhancement of the Raman scattering.

However, the generation of reliable SEROA spectra of biomolecules has been problematic due to difficulties in controlling spectral artefacts and low signal-to-noise ratios which complicate detection of true SEROA signals. Although several papers have presented possible SEROA spectra,[11,12] currently a proof demonstrating mirror image SEROA spectra from opposite enantiomers has not been reported. Recently, observation of SEROA spectra for the L- and D-enantiomers of cysteine has been claimed;[13] however the authors stated that no corresponding SERS spectra could be measured under the same conditions. Therefore, no surface enhancement had occurred in the earlier study,[13] either SERS or SEROA, with the observed spectral features probably being reflection-associated birefringent artefacts. Thus, SEROA has still not been confirmed as an experimental technique.

SEROA spectral features depend on SERS experimental conditions, since they reflect the stability of colloids over time periods longer than those typically used in conventional SERS.[14] Contributing factors, including the concentration of analyte and aggregating agents, pH, type of colloid and time dependence, have been studied in order to determine the effects of these parameters on SERS spectra,[15–17] as they should also be optimal for measuring SEROA.[18]

However, it has proven difficult to stabilise the extent of aggregation in colloidal systems sufficiently to control the fluctuation of bands in SEROA experiments, complicating validation of observed spectral features. Etchegoin *et al.* modelled the effect of surface plasmons on circularly polarized light.[19] Their calculations suggest that large artefacts in SEROA spectra would be highly sensitive to the nature of colloid–colloid interactions, explaining the origin of the intense and fluctuating features often observed in SEROA experiments. Slowing down changes in the aggregation state to minimize changes in colloidal interactions should improve the reliability and reproducibility of SEROA spectra.

In this study we employ a hydrophilic polyacrylic acid "polycarbopol" polymer as a stabilising medium. This polymer has small Raman and surface-enhanced Raman cross sections, minimising interference from background signals, does not significantly change the UV-vis absorption spectra when added to silver colloids and is known stabilise even aggregated colloids for extended periods of time.[20,21] We report SERS and SEROA spectra, along with the Raman and ROA spectra, of D- and L-ribose measured in the presence of citrate-reduced silver colloids and the polycarbopol polymer, providing the first definitive observation of SEROA.

Silver nitrate (99%), sodium borohydride (99%), sodium citrate (99%), sodium hydroxide (99%), potassium sulfate (99%), D- and L-ribose (99%) were purchased from Sigma-Aldrich UK and used without further purification. Citrate-reduced silver colloids were prepared by reduction of silver nitrate with citrate ions,[22] see ESI‡ for details. The polycarbopol polymer was purchased from B.F. Goodrich Ltd and used without further purification to form the

[a] *Faculty of Life Sciences, Manchester Interdisciplinary Biocentre, The University of Manchester, 131 Princess Street, Manchester, UK M1 7DN. E-mail: E.Blanch@manchester.ac.uk; Fax: +44 (0)161 236 0409; Tel: +44 (0)161 306 5819*
[b] *School of Chemistry and Chemical Engineering, The Queen's University of Belfast, Belfast, UK BT9 5AG. E-mail: s.bell@qub.ac.uk; Fax: +44(0) 2890 976524; Tel: +44(0)2890 974470*

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polymer-sol mixture. The Raman ($I_R + I_L$), scattered circularly polarized (SCP) ROA ($I_R - I_L$), SERS ($I_R + I_L$) and SCP SEROA ($I_R - I_L$) spectra were all measured using a ChiralRAMAN SCP spectrometer (BioTools Inc., Jupiter FL) operating in the backscattering configuration at an excitation wavelength of 532 nm with spectral resolution of 7 cm^{-1}. Raman and ROA spectra were taken with laser power of 0.625 W at the sample with data collection times of 4–6 h. The laser power for SERS and SEROA was 0.25 W at the sample with data collection times of 35 min. The details of sample, aggregating agent and colloid concentration are given in each figure legend. All SERS samples were prepared to 1 ml, the sample was left to sit for 15 min in order to obtain maximum SERS enhancement, which was determined from time dependence measurements, and then 20 mg of polycarbophil polymer powder was added and stirred vigorously for a few seconds, then left for 60 min in order to allow full hydration and swelling of the polymer prior to data collection.

The Raman and ROA spectra in aqueous solution obtained for D- and L-ribose are shown in Fig. 1. All spectra (Raman, ROA, SERS and SEROA) presented in this study are raw data without any smoothing, baselining, normalization or any other data pretreatment. The Raman and ROA band assignments for both enantiomers of ribose are summarized in Table S1 in ESI‡.[23–25]

The Raman and ROA spectra of D-ribose are in excellent agreement with those reported by Wen *et al.*[23] and those measured recently by Dr C. Johannessen in Glasgow (personal communication). We have repeated the Raman and ROA spectra for L-ribose, but these have not been previously reported. Mirror image responses are observed for most ROA bands, though it is not known why no ROA band appears near 877 cm^{-1} for L-ribose, though this spectrum is reproducible.

Fig. 2A and E presents the Raman and ROA spectra, respectively, of polycarbopol in solution, measured at the same concentration as used in the SEROA experiments, with SERS and SEROA spectra of D- and L-ribose shown in Fig. 2C, D, G and H, respectively, before and after addition

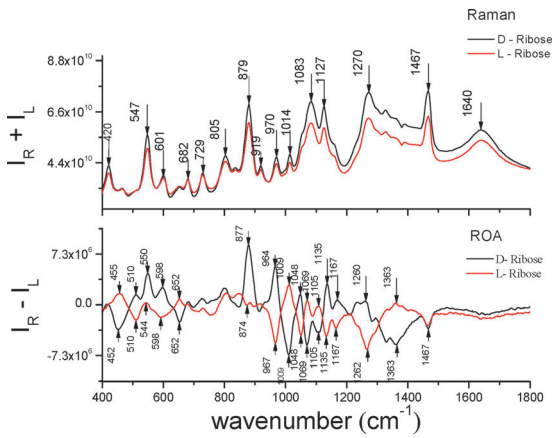

Fig. 1 Raman ($I_R + I_L$, top) and SCP ROA ($I_R - I_L$, bottom) spectra of D- and L-ribose in aqueous with their corresponding chemical structures above. Sample concentrations were 2.66 M at pH 5.46 (D-) and 5.60 (L-ribose), data collection time was 237.02 min, and laser power at the sample 0.625 W for each.

of polycarbopol polymer. The Raman spectrum of polycarbopol shows that the polymer does not generate any significant Raman signal as this polymer has a very small Raman cross section,[20] and only the spectrum of water is evident.

Although the polycarbopol subunit is chiral, it has a low Raman cross-section[21] so helping to minimise its ROA spectrum. Together, this leads to the ROA spectrum of polycarbopol being very weak, barely above the noise level. Fig. 2B and F shows spectra for the combination of silver colloids, aggregating salt and polycarbopol (no analyte). The two stronger bands at ~1394 and 1452 cm^{-1} in the SERS spectrum are a fingerprint of the sol with polycarbopol. The corresponding SEROA spectrum has negative features which are very noisy, that arise from both the polycarbopol and the interaction of plasmon resonances with circularly polarized light.

Fig. 2C and D presents the experimental SERS spectra of D- and L-ribose before and after, respectively, the addition of the polymer. The SERS spectra for D- and L-ribose shown in Fig. 2C and D were obtained using the optimum type of aggregating agent (K_2SO_4), its concentration (20 mM) and pH (8.7). The optimum concentration of the polycarbopol polymer was found to be 20 mg ml^{-1} which generated a viscous solution that was dilute enough to pipette but thick enough to control the aggregation of colloids, and gave rise to strong SERS signals for an extended period of time. The spectra demonstrate that the SERS signals for the two enantiomers are similar both in the presence (Fig. 2D) and the absence (Fig. 2C) of the polymer. All bands measured in the conventional SERS experiments appear at the same position in the presence of the polymer with only small differences in relative intensities of bands, confirming that the polymer does not interfere with signals from ribose molecules. Time dependence measurements, see Fig. S3 in ESI‡, show that SERS intensity is stable for over 35 min with the polymer, but for only 10 min without polymer. We conclude, therefore, that the addition of the polymer increases the stability of the aggregated colloids, allowing measurement of reliable SERS signals from the analyte.

The SEROA spectra of D- and L-ribose measured in the absence of polymer are shown in Fig. 2G. These spectra present a common problem that can occur in attempts to measure SEROA spectra. The SEROA spectrum of D-ribose gives rise to a mix of +ve and −ve bands, which are what may be expected in a chiroptical measurement, but in the spectrum of L-ribose all of the bands are negative in sign, due to difficulties in controlling the highly birefringent background signal. Therefore, we do not observe a mirror image response in Fig. 2G for any of the purported SEROA bands generated by the two enantiomers due to the large birefringence generated by the surface plasmons from the aggregating colloids, making it difficult to have confidence in the reliability of either of these two spectra. Furthermore, though the SERS spectra presented in Fig. 2C, which are insensitive to this problem, could be reproduced many times, the corresponding SEROA spectra demonstrated poor reproducibility both from sample-to-sample and as a function of time.

Fig. 2H shows the SEROA spectra of D- and L-ribose with polycarbopol polymer. Both D- and L-ribose give highly

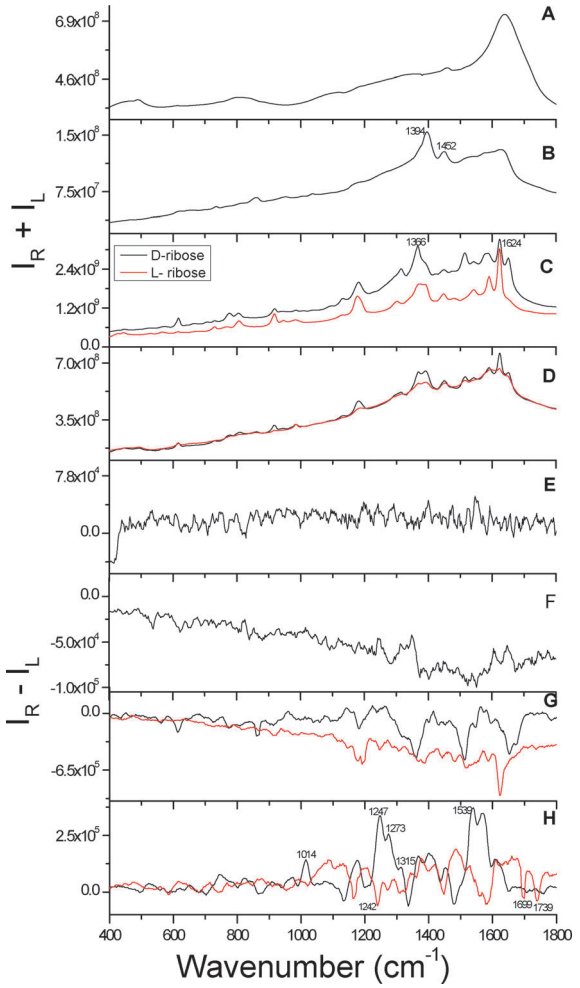

Fig. 2 Raman spectrum of polycarbopol in solution (A), SERS spectra before (B) and after addition of L- and D-ribose (0.25 mg ml^{-1}) in the presence of silver citrate reduced colloid and K_2SO_4 at 0.020 M concentration, data collection time: 20 min (C), SERS spectra of L- and D-ribose (0.25 mg ml^{-1}) in the presence of polycarbopol polymer, data collection time: 20 min (D), ROA spectrum of polycarbopol polymer in solution, sample concentration 40 mg ml^{-1}, data collection time: 218 min (E), SEROA spectra of silver citrate reduced colloids in presence of aggregating salt before (F) and after addition of L- and D-ribose, data collection time: 35 min (G), SEROA spectra of L- and D-ribose with addition of polycarbopol, data collection time of 35 min (H).

reproducible SEORA spectra (see Fig. S4 in ESI‡ for replicate measurements) with positive and negative bands. Critically, despite baseline variations, mirror image bands are now observed for the two enantiomers. The SEROA spectrum of D-ribose displays a number of bands that clearly show the opposite sign to their L-ribose counterparts. The +ve SEROA bands for D-ribose at 1247, 1273 and 1315 cm^{-1} correspond to the −ve SEROA bands for L-ribose at 1242, 1270 and 1310 cm^{-1}, respectively. A complex −ve/+ve/−ve triplet exhibited by D-ribose from ~1100–1230 cm^{-1} is nicely replicated as a +ve/−ve/+ve triplet by L-ribose with similar band shapes and intensities, as is the +ve/−ve couplet for D-ribose from ~1430–1500 cm^{-1}. A strong +ve SEROA band at 1571 cm^{-1} for D-ribose gives rise to an equivalent −ve feature for L-ribose. The regions between 1300–1400 cm^{-1} and below 1000 cm^{-1} reveal a number of weak features that appear to show opposite sign for the two enantiomers, though variations in local baselines due to residual birefringent background signals complicates their analysis.

However, several features in the SEROA spectrum for D-ribose do not lead to a mirror image for the L-enantiomer, most notably the +ve bands at ~1014 and 1539 cm^{-1}, while there are also no counterpart features to the −ve SEROA bands displayed by L-ribose at ~1699 and 1739 cm^{-1}. The reasons for these differences are not known, but they are reproducible (Fig. S4, ESI‡) so do not originate from variable birefringent artefacts or shot noise.

We have demonstrated the first experimental proof of SEROA by recording SEROA spectra for two enantiomers, D- and L-ribose, along with their corresponding SERS and ROA spectra. Addition of the polycarbopol polymer provides a solution to the problem of how to stabilize the aggregated colloids, and so reduce the effect of plasmon resonance-induced changes in circularly polarized light that typically plague SEROA experiments. This strategy will allow the potential of SEROA to be more effectively explored.

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